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Review

Nuclear bodies in neurodegenerative disease

John Woulfe *

Department of Pathology, The Ottawa Hospital, Civic Campus, 1053 Carling Avenue, Ottawa, Ontario, Canada K1Y 4E9
Departments of Pathology and Biochemistry, Microbiology, and Immunology, University of Ottawa, Canada

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ABSTRACT

Neurodegenerative diseases are characterized by a relentlessly progressive loss of the functional and structural integrity of the central nervous system. In many cases, these diseases arise sporadically and the causes are unknown. The abnormal aggregation of protein within the cytoplasm or the nucleus of brain cells represents a unifying pathological feature of these diseases. There is increasing evidence for nuclear dysfunction in neurodegenerative diseases. How this relates to protein aggregation in the context of "cause and effect" remains to be determined in most cases. Co-ordinated nuclear function is predicated on the activity of distinct nuclear subdomains, or nuclear bodies, each responsible for a specific function. If nuclear dysfunction represents an important etiopathological feature in neurodegenerative disease, then this should be reflected by functional and/or morphological alterations in this nuclear compartmentalization. For most neurodegenerative diseases, evidence for nuclear dysfunction, with attendant consequences for nuclear architecture, is only beginning to emerge. In this review, I will discuss neurodegenerative diseases in the context of nuclear dysfunction and, more specifically, alterations in nuclear bodies. Although research in this field is in its infancy, identifying alterations in the nucleus in neurodegenerative disease has potentially profound implications for elucidating the pathogenesis of these disorders.

1. Introduction

Neurodegenerative diseases are relentlessly progressive disorders of the central nervous system characterized by cognitive, motor, and/or behavioural dysfunction. This clinical heterogeneity is in large part attributable to pathological variability and to the characteristic topographic pattern of CNS involvement displayed by each particular disease entity, the latter being determined by selective vulnerability of populations of brain cells to the disease process. Abnormal protein aggregation represents a unifying biochemical and histomorphological hallmark of neurodegenerative disease [1]. The classification of neurodegenerative diseases is increasingly becoming based on the identity of the protein which accumulates (Table 1). In most cases, these protein aggregates form microscopically-visible cellular deposits called "inclusion bodies". In the majority of cases, these inclusion bodies are located in the cytoplasmic compartment. Accordingly, studies of disease pathogenesis have historically focused on aspects of aberrant protein biochemistry occurring in this part of the cell. The discovery that protein aggregation within the nuclear compartment represented a pathological hallmark of Huntington's disease [2,3], inculcated the nuclear compartment into the cast of cellular players known to contribute to neurodegenerative pathogenesis. Aberrant nuclear structure, function, or both, are emerging as important pathogenetic mediators in a growing number of neurodegenerative diseases [4]. For example, aberrant nucleocytoplasmic transport of transcription factors resulting in their abnormal cytoplasmic accumulation is increasingly recognized in several neurodegenerative disorders [5]. Moreover, in some diseases with primary nuclear involvement, for example the RNA-mediated disease myotonic dystrophy, the histomorphological changes in the nervous system are reminiscent of those described for more common sporadic neurodegenerative conditions, such as Alzheimer's disease (AD) [6]. This capacity for nuclear dysfunction to induce Alzheimer-like changes in the brain makes it tempting to invoke a nuclear contribution to the pathophysiology of AD and other common sporadic neurodegenerative conditions.

The concept of structural and functional compartmentalization within the nucleus is well-established [7]. Thus, individual nuclear bodies have been ascribed particular functional roles. These nuclear bodies include those most relevant to the discussion below such as Cajal bodies (or coiled bodies; CBs) and gemini of coiled bodies or "gems", both involved in small nuclear ribonucleoprotein (snRNP) metabolism and synthesis of the spliceosome [7], PML bodies involved in a variety of intranuclear functions [8], and splicing speckles or interchromatin granule clusters which represent sites of pre-mRNA splicing [9]. There is a growing list of additional nuclear bodies including cleavage bodies, matrix-associated deacetylase bodies, HAP bodies, nucleolar paraspeckles and clastosomes. The structure and function of nuclear bodies are reviewed in greater detail in the other reviews provided in this special issue. An important corollary of this nuclear compartmentalization is that disease-associated nuclear

* Tel.: +1 613 798 5555x13345; fax: +1 613 761 4199.
E-mail address: jwoulfe@ottawahospital.on.ca.

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Table 1
Protein-based classification of neurodegenerative disease

Tauopathies
Alzheimer's disease
Amyotrophic lateral sclerosis/Parkinson-dementia complex
Argyrophilic grain disease
Corticobasal degeneration
Dementia pugilistica
Diffuse neurofibrillary tangles with calcification
Down's syndrome
Frontotemporal dementia/parkinsonism linked to chromosome 17
Gerstmann–Straussler–Scheinker disease
Guadeloupian parkinsonism
Myotonic dystrophy
Niemann-Pick disease type C
Pick's disease
Postencephalitic parkinsonism
Progressive subcortical gliosis
Progressive supranuclear palsy
Subacute sclerosing panencephalitis
Tangle only dementia
Synucleinopathies
Parkinson's disease
Dementia with Lewy bodies
Multiple system atrophy
Neurodegeneration with brain iron accumulation
Pure autonomic failure
Meige's syndrome
Prion Proteinipathies
Creutzfeld–Jakob disease (familial, sporadic, and transmitted)
Fatal insomnia (familial and sporadic)
Gerstmann–Straussler–Scheinker disease
Kuru
Polyglutaminopathies
Huntington's disease
Dentatorubropallidoluysian atrophy
Spinal and bulbar muscular atrophy (Kennedy's disease)
Spinocerebellar ataxia 1, 2, 3, 6, 7, and 17
TDP-43 proteinopathies
Frontotemporal lobar degeneration with ubiquitin-only inclusions
Amyotrophic lateral sclerosis
RNA-mediated diseases
Fragile X-associated tremor ataxia syndrome
Myotonic dystrophy
Spinocerebellar ataxias 8, 10, 12
Huntington's disease-like type 2
Others
Neuronal intermediate filament inclusion body disease
Neuronal intranuclear inclusion disease
Neuroferritinopathy
TDP-43-negative FTLD-U

dysfunction should be manifest morphologically as alterations in the nuclear bodies whose functions are disrupted by the disease process. Thus, in acute promyelocytic leukemia (APL), the pathogenic *t* (15;17) chromosomal translocation results in a chimeric PML-RARα fusion protein with attendant disruption of intranuclear PML bodies [10,11]. Interestingly, the clinical response to treatment with ATRA correlates with relocalization of PML to PML bodies [11,12]. The concept of a similar involvement of nuclear bodies in neurodegenerative disease is beginning to emerge.

The focus of this review will be on neurodegenerative disease afflicting the central nervosus system. Thus, diseases primarily affecting muscle for which alterations in nuclear bodies have been described, (for example fascioscapulohumeral muscular dystrophy [13]), will not be discussed in great detail. The first section concerns difficulties in interpreting the cause-and-effect status of nuclear morphological alterations in the context of disease pathogenesis. Spinal muscular atrophy will then be discussed as an archetypal example of a neurodegenerative disease in which nuclear body alterations have been directly implicated in disease pathogenesis. For adult neurodegenerative diseases, the majority of studies concerning nuclear bodies thus far have focused on how they interact with, and are affected by, microscopically-visible, proteinaceous, pathological nuclear inclusion bodies. Therefore, I will discuss alterations in nuclear bodies in the "nuclear inclusion diseases", concentrating on the polyglutamine (polyQ) repeat disorders. Finally, the discussion will focus on nuclear dysfunction and known or potential nuclear body alterations in the more common, "canonical" adult neurodegenerative diseases including Alzheimer's disease, Parkinson's disease, frontotemporal dementia, and amyotrophic lateral sclerosis.

2. Generalized nuclear changes in neurodegenerative disease

In some neurodegenerative diseases, there is evidence that reorganization of nuclear compartments may reflect a primary intranuclear pathogenetic event. However other nuclear alterations may represent generalized consequences of the neurodegenerative process with, nonetheless, important pathogenetic and therapeutic implications. For example, a variety of environmental stress conditions, some of which may occur along the neurodegenerative cascade, have been demonstrated to result in marked nuclear body alterations. Examples include amino acid starvation, high temperature, osmotic changes, exposure to heavy metals, and cellular senescence [11]. There is growing evidence for generalized involvement of nuclear foci containing elements of the DNA-damage/repair mechanism in several neurodegenerative diseases [14]. This may be associated with a number of nuclear morphological alterations. Germane to this discussion are the observations of Valero and co-workers in mitral cells of the Purkinje cell degeneration (pcd) mutant mouse [15]. These mice harbour a recessive mutation in the *nna1* gene which encodes a nuclear protein involved in neuronal differentiation and regeneration [16]. They undergo selective degeneration of specific neuronal populations, including mitral cells in the olfactory bulb. In the latter, the authors described the formation of abnormally enlarged DNA-damage/repair foci containing the phosphorylated form of the histone variant H2AX, a sensor of DNA damage, as well as phosphorylated ataxia telangiectasia mutated (ATM) protein, a protein kinase that responds to DNA damage. This was associated with evidence of transcriptional downregulation with a reduction in the number and intensity of acetylated histone H4 and RNA polymerase II foci, the formation of large perinucleolar masses of trimethylated histone, an increase in the size and number of heterochromatin masses, and segregation of nucleoli. Most importantly in the context of this review was the finding that nuclear bodies, specifically coiled bodies (CBs) and splicing speckles, were disrupted. Both coilin and survival motor neuron protein (SMN) were re-distributed from the typical CBs seen in control cells to perinucleolar caps. Splicing factors of pre-mRNAs were re-organized into large, irregular nuclear speckles. Because these changes were observed in cells lacking morphological evidence of degeneration, they were considered a very early manifestation, or "predegenerative" in nature. The transcriptional and nuclear body alterations were proposed to reflect a dysfunction of gene expression and pre-mRNA processing, respectively, as a consequence of the accumulation of DNA-damage foci. Because there is increasing evidence that DNA damage may be a general feature of neurodegenerative disease, the secondary alterations in nuclear bodies as described in this study, must be distinguished from nuclear morphological alterations more proximate to the causative molecular lesion.

3. Spinal muscular atrophy

Spinal muscular atrophy (SMA) is a common autosomal recessive neurodegenerative disease characterized by progressive muscle weakness and wasting. It is the leading cause of inherited infant

mortality [17]. Pathologically, it is characterized by the loss of spinal cord anterior horn alpha motor neurons with consequent denervation of skeletal muscle. SMA is caused by deletion of the telomeric survival motor neuron 1 (*SMN1*) gene on chromosome 5q13 [18,19]. A centromeric (*SMN2*) copy of *SMN1* exists as an inverted duplication [20]. The coding sequence of *SMN2* differs from that of *SMN1* by a single nucleotide (840 C>T) which disrupts an exonic splicing enhancer and results in the exclusion of exon 7 from the *SMN2* transcript [21]. Thus, whereas the *SMN1* gene produces full length SMN protein (FL-SMN), the SMN2 gene produces predominantly exon7-deleted SMN, which is unstable and rapidly degraded, and a small amount of FL-SMN. The latter is not able to fully compensate for the loss of SMN1 but SMN2 can modify disease severity in a copy number-dependent manner [22]. How the depletion of FL-SMN protein culminates in the loss of motor neurons is unclear but alterations in nuclear bodies have been critically implicated in disease pathogenesis.

SMN functions as part of a large protein complex which includes, in addition to SMN itself, 7 additional proteins, the Gemins [23]. The function of the SMN complex is discussed in detail in a recent review [23]. Briefly, this complex is involved in the initial stages of spliceosome assembly by binding Sm proteins in the cytoplasm and transferring them as a heptameric ring on the Sm site of small nuclear RNAs to produce U snRNPs, the functional unit of the spliceosome. The spliceosome is responsible for the pre-messenger RNA splicing process which, in turn, is essential for the successful execution of eukaryotic gene expression and cell survival. In neurons, SMN protein is localized throughout the cytoplasm as well as in nuclei, where it is restricted to gems and to CBs. Gems are discrete, spherical nuclear bodies that have a size and shape similar to CBs [24]. Gems have been implicated in the pathogenesis of SMA. Thus, consonant with the depletion of SMN, gems are lost from the nuclei of motor neurons of SMA patients and the extent of gem loss correlates with disease severity [19,25]. Dysfunctional targeting of SMN to nuclear foci is a cardinal feature of SMA [26]. Most SMA causing mutations result in a truncated form of the protein that lacks sequences critical for self-oligomerization [27] and proper translocation to the nucleus, both necessary for gem formation.

Nuclear bodies do not function in isolation but show dynamic spatial interactions with one another reflecting a highly co-ordinated intranuclear functional network. Accordingly, pathological alterations affecting one type of nuclear body should have consequences for the nuclear bodies with which they interact. Experimental studies suggest that this is the case for Gems and CBs in SMA [26]. The tight association between these two nuclear bodies has raised questions regarding whether they are truly separate entities [28]. SMN and p80 coilin, the principal protein constituent of CBs, have very similar intranuclear localization patterns in neurons, including localization to perinucleolar caps [24]. However, in some cells and under some conditions, p80 coilin and SMN label distinct structures [28]. Gems, per se, reportedly do not contain snRNP whereas CBs do [24]. Regardless of whether they are distinct structures, it is clear that there are molecular interactions between the two. The recruitment of SMN to nuclear bodies is dependent on p80 coilin, indicating that coilin provides a molecular bridge between CBs and gems [26]. The functional inter-relationship between Gems and CBs is reflected by alterations in the latter under conditions of SMN depletion. Thus, Girard and co-workers [29] described loss of canonical CBs in SMN-depleted Hela cells with the formation of multiple small coilin foci and localization of coilin to the nucleolus. These coilin foci did not contain snRNPs. Frugier et al. [30] also provided evidence for defects in CB assembly in motor neurons of a mouse model of SMA in which mutant SMN lacking the *carboxy*-terminus was targeted to neurons. Immunostaining for p80 coilin revealed loss of typical CBs and the formation of large intranuclear aggregates.

The involvement of CBs and coilin in the pathogenesis of SMA has important implications for our understanding of the potential involvement of the nucleus not only of this disease, but other neurodegenerative diseases as well. As speculated by Matera and Frey [28], it could be interpreted to suggest that aberrations in other aspects of the snRNP pathway (for example p80 coilin mutations), by analogy with SMA, could also have neurodegenerative phenotypes.

Table 2
The neuronal intranuclear inclusion neurodegenerative diseases

Disease	Gene/protein	Neurons	Glia	HE	Ubiq	PML	1C2	NI localization in nervous system
Unstable repeat diseases								
Protein-mediated (PolyQ)								
HD	*HD (IT15)/huntingtin*	N>C20, 21	No		Yes	Yes**	Yes	ctx, str
DRPLA	*DRPLA/atrophin 1*	N>C25, 30	N25, 37	Yes25	Yes	Yes	Yes	ctx, gp, str, cb ctx, stn, rn, dn
SBMA	*AR/androgen receptor*	N32	No		Yes	?	Yes	spinal and bulbar motor neurons
SCA1	*SCA1/ataxin 1*	N23, 24	C29		Yes	Yes*	Yes	pons, ion
SCA2	SCA2/ataxin 2	C>N34, 40	C40		Yes	Yes	Yes	pons, sn, ion, thl, gp, lc, ctx
SCA3	*SCA3/ataxin 3*	N>C22, 36, 38	C38		Yes	Yes	Yes	pons, dn, sn, thl, nbM, lgn, ctx
SCA6	*CACNA1A/CACNA1A*	C>N35 N>>C3	No		No33	?	Yes	Pkj
SCA7	*SCA7/ataxin 7*	1	No		Yes	Yes	Yes	ctx, lgn, pons, ion, sn, Pkj, pons, raphe, thl, nbM, cb
SCA17	*SCA17/TBP*	N39	No	Yes27	Yes	Yes	Yes	ctx, ctx
Protein-mediated (PolyA)								
OPMD	*PABPNI/PABPNI*	N	No		Yes	Yes*	No	cb ctx
RNA-mediated								
DM1	*DMPK/DMPK*	N	No		No	?	No	hc, dg, thl, sn, Pkj
DM2	*ZNF9/ZNF9*	N/I	N/I		No	?	No	NI
FXTAS	*FMR1/FMRP*	N	N	Yes	Yes	Yes**	No	hc, ctx, bg, sn, thl, dn, ion, XII, ec's, cpc's
Others								
NIID FTD	Unknown	N	N	Yes	Yes	Yes	Yes/No	ctx, hc, bg, thl, cb, bs, sc, drg, pn
FTLD-U-NI	*PGRN/PGRN*	C>N	N		Yes	Yes	No	ctx, dg, str
IBMPFD	*VCP/VCP*	N>C	N		Yes	Yes**	No	ctx, hc, str, gp
NIFID	Unknown	C>N	No	Yes	Yes	?	Yes/No	ctx, hc, dg, amyg, str, gp, thl, rn, sn, pons, ion,Pkj
MSA	Unknown	C>N	C>N		Yes	?	No	widespread; bg, cb, pons, ion, imlcc
Neuroferritinopathy	*FTL/FTL*	N>C	N>C	Yes	Yes	?	No	str, gp, ctx, cb ctx, pn, ic, ac, cpc's

References have been supplied for items not discussed in the text. * denotes evidence from experimental studies only. ** denotes evidence from author's laboratory. Abbreviations not in text: XII, hypoglossal nucleus; ac, anterior commissure; amyg, amygdala; bg, basal ganglia; bs, brainstem; C, cytoplasmic; CACNA1A, calcium channel, voltage-dependent, P/Q type, a1A subunit; cb, cerebellum; cb ctx, cerebellar cortex; cpc's, choroid plexus epithelial cells; ctx, cerebral cortex; dg, dentate gyrus; dn, dentate nucleus; drg, dorsal root ganglia; ec's, ependymal cells; gp, globus pallidus; hc, hippocampus; ic, internal capsule; imlcc, intermediolateral cell column; ion, inferior olivary nucleus; IT15, interesting transcript 15; lc, locus coeruleus; N, nuclear; nbM, nucleus basalis of meynert; NI, not investigated; Pkj, Purkinje cells; pn, peripheral nerve; rn, red nucleus; sc, spinal cord; sn, substantia nigra; stn, subthalamic nucleus; str, striatum; thl, thalamus. Reprinted with permission from: Woulfe JM, Abnormalities of the nucleus and nuclear inclusions in neurodegenerative disease: a work in progress. Neuropathology and Applied Neurobiology 2007;33:2–42. Wiley-Blackwell publishing.

4. The "nuclear inclusion diseases"

A number of neurodegenerative diseases are characterized pathologically by the presence of microscopically-visible, proteinaceous intranuclear inclusion bodies (NIs) within neurons and, less commonly, glial cells. The mere presence of these imposing structures within the nucleus has led to investigations of how they might interact with, or disrupt the function of, nuclear compartments. The "nuclear inclusion" diseases comprise a large family including the polyglutamine (polyQ) repeat diseases, the polyalanine (polyA) repeat diseases, the RNA-mediated diseases, subtypes of frontotemporal dementia, neuronal intranuclear inclusion disease, multiple system atrophy, and neuroferritinopathy (see Table 2). A complete discussion of the clinical features, pathological features, and pathogenesis of these disorders is the subject of a previous review [4]. The NIs consist of aggregation-prone mutant proteins (in the case of hereditary diseases) or otherwise post-translationally altered/misfolded proteins (in the case of sporadic diseases), or expanded RNA and RNA-binding proteins (in the case of the RNA-mediated diseases). In the case of the polyQ repeat diseases, an unstable CAG (the codon for glutamine) trinucleotide repeat expansion mutation in the affected gene leads to the production of a mutant protein with a long expansion of glutamine (Q) residues. This polyQ tract confers on the protein a toxic gain-of-function and results in the appearance of NIs consisting of ubiquitinated insoluble mutant polyQ protein. The role these inclusions play in causing neuronal degeneration is uncertain. The prevailing theory posits that the formation of visible filamentous intranuclear inclusion bodies is a protective mechanism to sequester more soluble, toxic oligomeric or protofibrillar forms of the pathogenic protein in an intranuclear "safehouse" [31]. On the other hand, the ability of NI's to bind functionally important nuclear proteins, including transcription factors, components of the ubiquitin-proteasome system and, in the case of the RNA-mediated diseases, RNA splicing factors, has engendered the concept that NI's effectively deplete the nucleus of these proteins with consequent transcriptional, degradative, and splicing dysregulation. Of these, transcriptional dysregulation has received the most attention as a potential pathogenic mediator [32,33].

The influence of NI's on nuclear body organization and function has been most extensively studied with respect to PML bodies. These spherical, 0.1 to 2 μm diameter bodies have been implicated in transcriptional regulation, growth suppression, apoptotic cell death, DNA repair, intranuclear protein storage and proteolysis, and in the controversial phenomenon of intranuclear protein translation [8]. PML, the signature protein of PML bodies has een shown to localize to NIs in a number of polyQ diseases [34–41] where it typically forms a ring, or shell around a ubiquitin-positive core. PML localization to NI's is not confined to polyQ diseases. We have observed PML staining of NIs in a variety of NI diseases (Fig. 1a–c).

As described by others [40,42], the association of PML with NIs is size-dependent whereby PML staining is confined to small NIs with larger ones being PML negative (Fig. 1c, d). This has been interpreted to suggest a model for PML-associated NI formation (Fig. 1e) which implicates "seeding" of mutant protein within pre-existing PML bodies [40–42]. Further protein recruitment leads to enlargement with eventual "obliteration" of the PML body. In this scenario, PML bodies represent sites of NI formation. Fu and co-workers provided evidence that large NIs form by fusion of smaller PML-associated NIs [43]. They demonstrated a marked re-distribution of PML bodies along the surface of NIs in the absence of changes in CBs or nuclear speckles. The NI-induced disruption of PML bodies may have pathogenic consequences. For example, the stress responses of PML bodies are ablated by mutant ataxin 1 (the protein that accumulates in nuclear inclusions in spinocerebellar ataxia type 1) and other nucleoprotein inclusions [44]. This is associated with loss of typical PML bodies from nuclei harbouring PML-immunoreactive NIs. Moreover, in the polyQ disease dentatorubropallidoluysian atrophy, there is altered abundance of RE repeats encoded protein, a protein normally localized to PML bodies [11]. In keeping with the purported proteolytic function of PML bodies, it is also conceivable that the size-dependent association of PML and NIs reflects successful intranuclear proteolysis of NI protein. PML is a ring finger protein with purported E3 ubiquitin ligase activity [45] and it could be involved in the degradation of abnormal

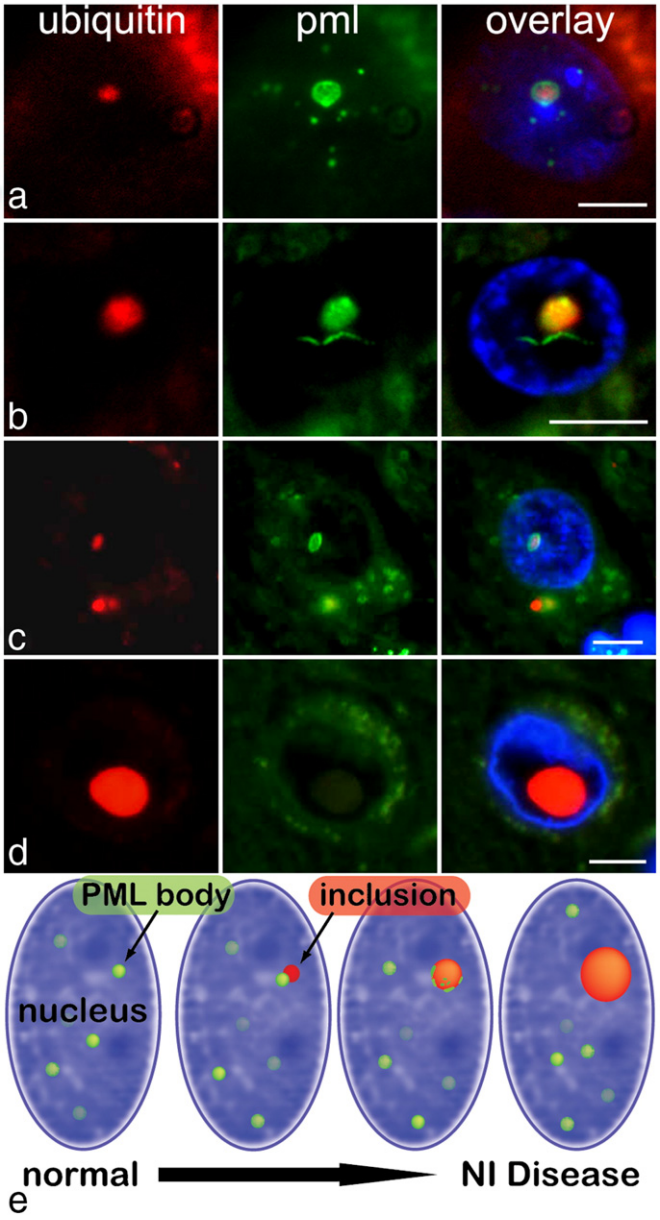

Fig. 1. PML staining of NIs. Double immunostaining for ubiquitin (red) and PML (green) of NIs in a cortical neuron in HD (a), an astrocyte in the pontine white matter in fragile X-associated tremor ataxia syndrome (b). Note multiple normal PML bodies in (a). Also note the linear PML body in the astrocyte in (b). c, d) A small ubiquitinated NI in a cortical neuron in neuronal intranuclear inclusion disease is PML-immunoreactive (c) whereas a large NI in the same case is negative for PML (d). This size-dependency of PML positivity inspired the model depicted in (e) whereby NIs seed in PML bodies. As more protein accumulates, the NI outgrows the PML body, culminating in the formation of a large NI lacking PML immunoreactivity. Bars=5 μm. Tissue from HD case in a provided by Dr. Yves Robitaille. Tissue from FXTAS case in b provided by Dr. Marc DelBigio. Illustration in (e) by Dr. Douglas A. Gray. Reprinted with permission from: Woulfe JM, Abnormalities of the nucleus and nuclear inclusions in neurodegenerative disease: a work in progress. Neuropathology and Applied Neurobiology 2007;33:2–42. Wiley-Blackwell publishing.

intranuclear proteins. Takahashi-Fujikasaki and co-workers [42] themselves described "empty large nuclear bodies" showing ring-like PML staining surrounding a polyQ-negative core. They interpreted these changes in staining properties as successful proteolysis of polyQ protein in pre-existing NIs. There could be therapeutic implications in elucidating whether: 1) PML nuclear bodies are involved in the formation or degradation of NIs and 2) NIs are toxic or protective. A variety of environmental or pharmacological manipulations cause alterations in PML bodies [46]. These manipulations might someday be exploited to enhance or, alternatively, mitigate NI formation.

Alterations in CBs in *neurodegenerative* disease have been less extensively scrutinized. These subnuclear domains play a critical role in the biogenesis of snRNPs, integral components of the pre-mRNA splicing machinery, and splicing aberrations have been implicated in a number of neurodegenerative diseases. CBs are not affected by the presence of NIs composed of polyQ-expanded ataxin 1 [34], but are closely associated with the NIs in the polyQ diseases dentatorubro-pallidoluysian atrophy and spinocerebellar ataxia type 3 [38]. Sun and co-workers [47] demonstrated that NIs composed of mutant ataxin-3, but not ataxin-1 or huntingtin, were "tethered" to CBs in vitro confirming a differential effect of distinct polyQ proteins on CB organization. Moreover, mutant ataxin-3 was able to disrupt the splicing of an artificial reporter, providing evidence for the pathogenic potential of the NI-CB interaction. The mechanism for this functional effect remains uncertain since the association frequency of CBs with U2 genes was not altered. This work calls into question the prevailing theory that NIs in polyQ neurodegenerative diseases are all protective.

Of potential interest to neurodegenerative disease are the SUMOylation bodies recently described by Navascues and co-workers in a PC12 neuron-like cell line [48]. SUMOylation is a molecular regulatory pathway involving the covalent attachment of the small ubiquitin-like modifier (SUMO) to lysine residues [49]. It is emerging as an important mechanism of transcriptional control [50,51] and as a crucial player in neurodegenerative pathophysiology [52]. SUMO-1 nuclear bodies (SNBs) are 1 to 3 μm in diameter, are free of PML protein, splicing factors, and transcription foci, and show spatial association with CBs. They concentrate the transcriptional regulators CBP, CREB and c-*jun*. By compartmentalizing them, SNBs may participate in the control of the nucleoplasmic concentration of transcriptional regulators involved in neuroprotection and survival [48]. SUMO modification has been implicated in human neurodegenerative disorders [53]. NIs in neuronal intranuclear inclusion disease contain SUMO-1 and the SUMOylation substrates PML, histone deacetylase 4, and RanGap1, suggesting that recruitment of SUMO-1 modified proteins into NIs may contribute to disease pathogenesis [42,54]. We have demonstrated SUMO-1 immunoreactivity of NIs in a variety of NI neurodegenerative diseases (Fig. 2). Aberrant SUMOylation has also been implicated in neurodegenerative diseases without obvious nuclear morphological changes (for an excellent review see [52]).

The unique SUMO-containing PML nuclear bodies described in neurons of the supraoptic nucleus [55] and the substantia nigra (Fig. 3) [56] may represent an SNB equivalent in the human brain. It will be interesting to determine how these structures are altered in neurodegenerative disease.

5. Nuclear bodies in common neurodegenerative diseases

Until recently, alterations in nuclear structure and function have been relatively neglected in more common neurodegenerative disorders like Alzheimer's disease (AD), Parkinson's disease (PD), frontotemporal dementia (FTD), and amyotrophic lateral sclerosis (ALS) perhaps because these diseases lack obvious nuclear morphological changes. Instead, cytoplasmic or extracellular protein aggregates represent the histopathological hallmarks of these diseases. Indeed, in rare families, causative mutations in genes encoding the aggregated proteins attest to the importance of these proteins in disease pathogenesis. However, the vast majority of cases are sporadic, with no evidence of mutations in the genes for these aggregating proteins. The pathophysiological mechanisms leading to protein aggregation in sporadic forms of these neurodegenerative diseases are unknown. However, evidence is accumulating that risk factors for these diseases may lie within the confines of the nuclear membrane; involving alterations at the level of gene expression and splicing, or mediated by "polymorphisms" in regulatory regions of the genes encoding disease-relevant proteins [57,58]. Moreover, the abnormal localization of nuclear transcription factors may represent a common feature of a variety of neurodegenerative disorders [5]. To what extent is this nuclear involvement reflected by nuclear morphological changes and, more specifically, alterations in nuclear bodies? Ultrastructural investigations of AD have revealed irregularities in the nuclear envelope including fragmentation, prominent nuclear pore aggregation, and a close association with paired helical filaments [59,60]. Some studies have reported the presence of NIs in AD [61]. Toper and co-workers [62] reported intranuclear rodlets, spherical inclusion bodies, and "vermicellar-like" inclusions. However, how these nuclear functional alterations are reflected in, or alternatively, are consequent upon, changes in nuclear bodies, are in their infancy.

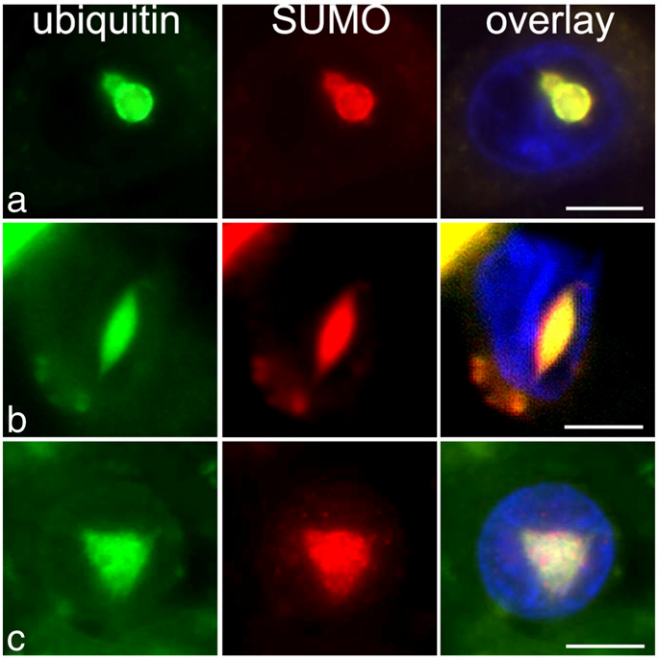

Fig. 2. SUMO staining of NIs. NIs in cortical neurons in cases of neuronal intranuclear inclusion disease (a), FTLD-U (b), and IBMPFD (c) double-immunostained for ubiquitin (green) and SUMO-1 (red). Bars = 5 μm. Tissue from neuronal intranuclear inclusion disease and FTLD-U-NI cases in a, b provided by Dr. Ian Mackenzie. Tissue from IBMPFD case in c provided by Dr. Mark Forman. Reprinted with permission from: Woulfe JM, Abnormalities of the nucleus and nuclear inclusions in neurodegenerative disease: a work in progress. Neuropathology and Applied Neurobiology 2007;33:2–42. Wiley-Blackwell publishing.

5.1. Alzheimer's disease

Alzheimer's disease (AD) is a common, dementing, relentlessly progressive neurodegenerative disorder of unknown etiology [63]. The histopathological hallmarks of AD are extracellular deposits of β-amyloid protein in the cerebral cortex and intracellular deposits of hyperphosphorylated tau protein in the form of neurofibrillary tangles. There is strong evidence for a role for β-amyloid and tau protein in AD pathogenesis [64]. β-amyloid is generated by sequential proteolytic cleavage from a larger, type I transmembrane protein called amyloid precursor protein (APP), encoded on chromosome 21. This processing is accomplished by the actions of β-secretase and γ-secretase (reviewed in

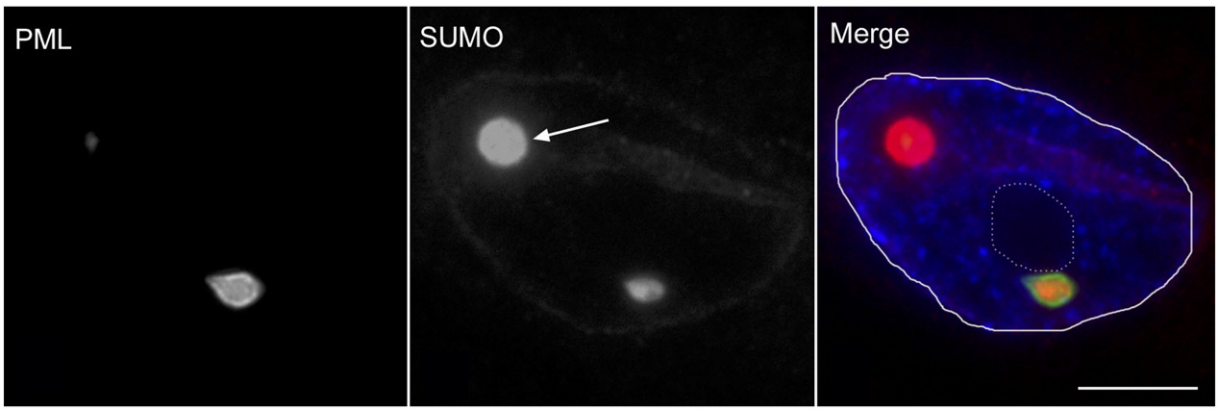

Fig. 3. SUMO-immunoreactive PML body and Marinesco body (arrow) within the nucleus of a human substantia nigra neuron double-immunostained for PML (green) and SUMO (red). Counterstained with DAPI (blue). The nuclear membrane is indicated with a solid line and the nucleolus with a dotted line. Note the juxtaposition to nucleoli (dotted line). Bar = 5 μm. Reprinted with permission from: Woulfe JM, Prichett-Pejic W, Rippstein P, Munoz DG. Promyelocytic leukemia immunoreactive neuronal intranuclear rodlets in human brain. Neuropathology and Applied Neurobiology 2007;33:56–66. Wiley-Blackwell publishing.

[65]). Recent evidence indicates a link between APP processing and changes in gene expression [66]. Thus, in addition to generating the pathogenic Aβ, these cleavage events generate a large amino terminal fragment of APP as well as a carboxy-terminal fragment, Cγ (reviewed in [64,65]). APP may signal to the nucleus via Cγ, which is transported to the nucleus in a complex with the adaptor protein Fe65 where it may regulate transcription through as yet undetermined mechanisms [67]. In addition to this role in transcriptional regulation, Cγ may have other nuclear roles. Muresan and Muresan demonstrated that a phosphorylated form of Cγ localizes to nuclear splicing speckles (but is excluded from CBs and Gems) [68]. They suggested that splicing speckle Cγ may have a role in pre-mRNA splicing. In this context, it would be interesting to know whether, and how, nuclear splicing speckles are altered in AD.

Indeed, there is evidence for transcriptional and post-transcriptional dysregulation in AD [69]. Total cellular RNA and polyA RNA are substantially reduced in the AD cortex [70]. At the post-transcriptional level, there are alterations in RNA-binding proteins [71]. This transcriptional dysregulation may involve CBP [72,73]. Reduced levels of CBP and the subsequent reduction in expression of CREB/CBP target genes have been found in familial AD [74]. A model of transcriptional dysregulation in familial AD based on impaired cleavage of transcriptionally-active, non-APP substrates, such as Notch-1 has been proposed [69,75].

Tau protein, the constituent protein of cytoplasmic neurofibrillary tangles, has also been implicated in AD pathogenesis [76]. Tau is a microtubule associated protein which promotes microtubule stability [77]. In AD, tau becomes hyperphosphorylated and forms neurofibrillary tangles. How hyperphosphorylated tau protein and/or NFTs damage neurons in AD is uncertain, but intranuclear mechanisms may be involved. The demonstration of intranuclear paired helical filaments (the ultrastructural counterpart of neurofibrillary tangles) in AD [59] is compatible with the presence of tau in the nucleus of normal neurons as well as non-neuronal cells [78–81]. Normal tau has DNA binding capacity and may protect DNA from denaturation [82]. Nuclear tau localizes to the internal periphery of nucleoli, partially co-localizing with nucleolin and directly binding with AT-rich α-satellite DNA sequences organized as constituitive heterochromatin [83]. Because perinucleolar heterochromatin and the associated proteins have an important role in the regulation of nucleolar structure [84], the latter authors proposed that nucleolar tau participates in the organization of the nucleolus and/or the heterochromatization pattern of a subset of RNA genes. In the setting of AD, hyperphosphorylated, aggregation-prone nuclear tau loses its capacity to interact with DNA [85] and could thereby disrupt nucleolar organization, or alternatively, the association of certain genes with the nucleolus. In this context, it is interesting that there is a lower frequency of satellite association in relation to chromosome 21 in AD, a provocative finding in light of the localization of the APP gene on this chromosome. Alterations in rRNA levels have been reported in AD [86,87]. However, studies of post-mortem AD brain tissue reveal no effect of neurofibrillary tangle formation, per se, on nucleolar size [88]. There are conflicting results regarding the size of nucleolar organizer regions in AD relative to age-matched controls with some studies showing a decrease [89] and others showing a (compensatory?) increase [90].

In AD, the formation of NFTs involves tau hyperphosphorylation [91]. In other tauopathies, the formation of NFTs results from aberrant mRNA splicing of exon 10 [92]. Glycogen synthase kinase-3 (GSK-3) is a key enzyme regulating tau metabolism and has been implicated in both mechanisms [93]. GSK-3 accumulates in the cytoplasm of neurons developing NFTs in AD and other tauopathies [94]. Interestingly, inhibiting GSK-3 in cultured neurons resulted in aberrant splicing of exon 10 and an enrichment of SC35 in nuclear splicing speckles where it co-localized with GSK-3 [95]. Moreover, nuclear GSK-3 phosphorylated SC35 protein. In this context, it is tempting to speculate that abnormalities in GSK-3 function or localization to nuclear splicing speckles might have a role in the pathogenesis of tauopathies not linked to mutations in the tau gene, or even to AD.

We have provided evidence for involvement of another, neuron specific, nuclear body in the pathogenesis of AD. Neuronal intranuclear rodlets (INRs) are rod-shaped intranuclear bodies present in the normal human brain where they show a widespread but anatomically heterogeneous pattern of distribution (Fig. 4a) [96]. They can be demonstrated in a variety of mammalian species as well as in neurons in vitro. The functional significance of these structures is unknown but their formation has been linked to changes in neuronal electrical or transcriptional/translational activity [97,98]. In our studies of the functional significance of INRs, we have demonstrated non-random spatial interactions with other nuclear bodies including nucleoli and PML bodies [56,99]. This is consistent with previous electron microscopic studies demonstrating spatial interactions between INRs and CBs [100]. We have demonstrated that neuronal INRs are significantly and markedly depleted in the temporal cortex of AD patients relative to controls and those with another common age-related neurodegenerative dementia, dementia with Lewy bodies [101]. We are currently investigating the functional and pathological significance of this phenomenon but, given our evidence for an interaction of INRs with other nuclear bodies, it is consistent with the data presented above for nuclear involvement in AD pathogenesis.

5.2. Parkinson's disease

Parkinson's disease is the most common neurodegenerative movement disorder and is characterized pathologically by a relatively selective loss of dopaminergic neurons in the substantia nigra. The vast

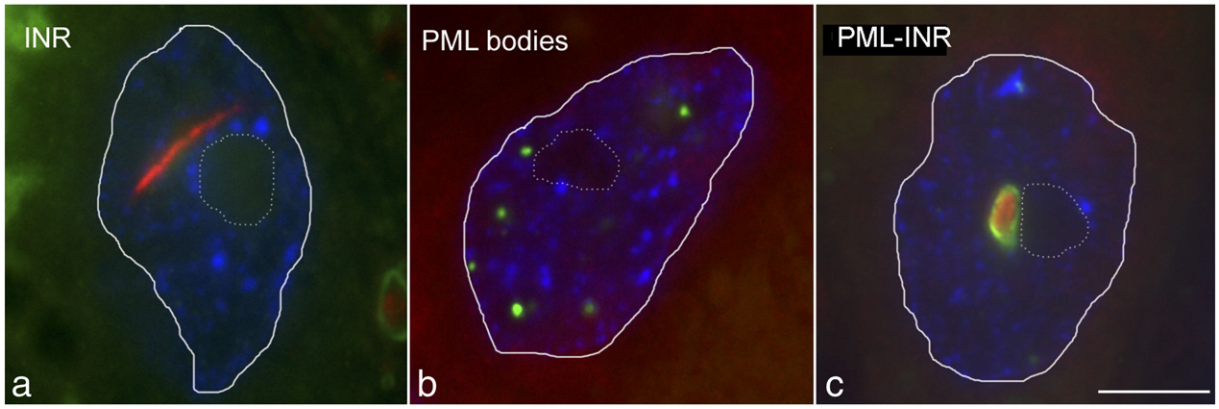

Fig. 4. Fluorescence microscopy images of human SN neurons double-immunostained for PML (green) and class III beta tubulin (C3T; red). Three types of immunostained structures are demonstrated including C3T+/PML-typical INR (a), C3T-/PML+ nuclear bodies (b) and C3T+/PML+ INR (c). The nuclear membrane is indicated by a solid line, and the nucleolus by a dotted line. Note the juxtanucleolar location in c. Bar=5 μm. Reprinted with permission from: Woulfe JM, Prichett-Pejic W, Rippstein P, Munoz DG. Promyelocytic leukemia immunoreactive neuronal intranuclear rodlets in human brain. Neuropathology and Applied Neurobiology 2007;33:56–66. Wiley-Blackwell publishing.

majority of cases are sporadic. Rare familial cases with autosomal dominant or autosomal recessive inheritance have provided clues regarding pathogenesis of the sporadic form of the disease. A protein called α-synuclein has emerged as a key player in disease pathogenesis [102]. Missense mutations in α-synuclein cause PD in rare kindreds. In others, duplications, or triplications, of the α-synuclein gene cause PD, indicating a role for α-synuclein dosage in causing the disease. Finally, in both familial as well as sporadic forms of the disease, α-synuclein is the principle protein constituent of Lewy bodies, the roughly spherical intracytoplasmic inclusion bodies that are the histopathological hallmark of PD. In normal neurons, α-synuclein localizes to presynaptic axon terminals and to the nucleus [103]. The majority of studies of this important protein have focused on it's role in the axon terminal. However, it's nuclear localization has been reported in a variety of species as well as in vitro [104,105]. Moreover, α-synuclein forms neuronal and glial intranuclear inclusions in patients with a neurodegenerative disorder known as multiple system atrophy [106] as well as in mice expressing human α-synuclein [107]. Synuclein translocates to the nucleus of mice injected with the PD-inducing herbicide paraquat and, once in the nucleus, can associate with histones, suggesting that the abnormal formation of histone-α-synuclein complexes may have relevance for PD pathogenesis [104]. Indeed, α-synuclein mutations that cause familial PD exhibit increased nuclear targeting in vitro [108]. The latter authors provided strong evidence that α-synuclein mediates neurotoxicity in the nucleus by demonstrating that targeting of α-synuclein to the nucleus promoted toxicity whereas cytoplasmic sequestration was protective. Moreover, α-synuclein bound directly to histones, reduced the level of acetylated histone H3, and inhibited acetylation in histone acetyltransferase assays. Remarkably, the toxicity of α-synuclein could be rescued by administration of HDAC inhibitors, revealing potential therapeutic relevance for aberrant intranuclear interactions of α-synuclein in PD.

Despite this impressive evidence for a pathogenic intranuclear role for α-synuclein, it's intranuclear distribution is described as diffuse [108] or "uneven with a reduced presence in nucleoli" [109] with no reference to any particular nuclear body. Moreover, whether nuclear bodies are re-organized or otherwise altered as a consequence of intranuclear α-synuclein dysfunction in PD remains to be investigated. Gertz and co-workers [110] found no significant difference in nucleolar size when comparing neurons with Lewy bodies and without Lewy bodies in PD brain, suggesting no influence of cytoplasmic α-synuclein aggregation on RNA synthetic activity. However, there was a significant negative correlation between nucleolar size and duration of disease.

Of potential relevance to PD and the substantia nigra are Marinesco bodies (MBs) and INRs. MBs are predominantly spherical eosinophilic intranuclear bodies found in pigmented neurons of the substantia nigra and locus coeruleus of the non-diseased human brain. Whether they represent nuclear bodies or "quasi-pathological" nuclear inclusions is a matter of debate. Like disease-associated NIs, MBs are immunoreactive for ubiquitin [111], ataxin-3 [112], PML [113], and SUMO-1 (Fig. 3). Interestingly, MBs are also SUMOylated and contain histone deacetylase 4 [114]. The frequency of MBs increases with age [115,116]. It has been postulated that MB formation is a response to cellular stress or to impaired handling of intranuclear protein [115–117]. The age-related increase in MBs has been implicated in the selective vulnerability of these neurons to degeneration in aging and in PD [118]. MBs are significantly increased in nigral neurons of patients with dementia with Lewy bodies and in Lewy body-bearing neurons [119]. In one study, MB frequency correlated significantly, and inversely, with markers of striatal dopamine in non-diseased elderly subjects [119]. We have demonstrated that INRs in dopaminergic neurons of the human substantia nigra show intimate, non-random spatial interations with MBs and postulated that MBs may arise from INRs or vice versa [99].

Injection of mice with the selective dopaminergic neurotoxin MPTP, results in the degeneration of neurons in the substantia nigra and represents a widely used animal model of PD [120]. We have demonstrated a significant upregulation in the number of INRs in dopaminergic neurons in MPTP-treated mice, suggesting possible involvement of this structure in PD pathogenesis [121]. These findings are intriguing because in dopaminergic neurons of the human substantia nigra, INRs show an interaction with PML and features not seen in other areas of the central nervous system (with the possible exception of the PML nuclear bodies in the supraoptic nucleus [55]). Here, in addition to typical rod-shaped INRs (Fig. 4a) and spherical PML bodies (Fig. 4b), there are roughly spherical to elongated, PML-immunoreactive INRs consistently associated with the nucleolus (Fig. 4c), and with MBs [99]. These show a central zone of tubulin or glucocorticoid receptor staining surrounded by a PML-immunoreactive ring (Fig. 4c), similar to the pattern described for disease-associated NIs [56]. A proportion also show immunostaining for ubiquitin, SUMO-1 (Fig. 3) and 20S proteasome [56]. Staining for the latter is interesting in light of a study demonstrating nuclear localization of the 20S proteasome in PD, but not control substantia nigra [122]. Curiously, a small proportion of INRs are immunoreactive for acetylated histone H4, indicating the presence of trancriptionally-active chromatin as well as for the eukaryotic translation initiation factor eIF4e. Finally, they show immunoreactivity for CBP. Whether these special features of INRs in dopaminergic neurons have

any relevance to their selective vulnerability in PD remains to be explored.

5.3. Frontotemporal dementia

Frontotemporal dementia (FTD) is a clinically, genetically, and pathologically heterogeneous group of diseases characterized by changes in personality and social behaviour with relative preservation of memory and perception [123,124]. A genetic basis for FTD is supported by the presence of a family history in approximately half of all cases [125]. Pathologically, FTD is characterized by varying degrees of frontal and temporal lobar degeneration. Two groups can be discerned on the basis of histopathology and immunohistochemistry: those featuring tau-immunoreactive cytoplasmic neurofibrillary inclusions (the tauopathies) and those lacking tau-positive inclusions (the non-tauopathies). The non-tauopathies account for 60% to 72% of all patients with FTD [123,126]. They include dementia lacking distinctive histopathology, frontotemporal lobar degeneration with ubiquitin-only inclusions (FTLD-U), frontotemporal dementia with motor neuron disease (FTD-MND), neuronal intermediate filament inclusion body disease (NIFID), and inclusion body myopathy/Paget's disease of bone/frontotemporal dementia (IBMPFD) [124,126–131].

FTLD-U is the most common type of FTD, comprising up to 62% of all patients and almost all patients with non-tauopathy FTD. Histopathologically, FTLD-U is characterized by the presence of ubiquitin-immunoreactive, tau- and synuclein-negative dystrophic neurites and neuronal cytoplasmic inclusions in neurons of the hippocampus and cerebral cortex [124]. The inclusions are similar to those described in motor neurons of amyotrophic lateral sclerosis (ALS) and, indeed pure motor neuron disease and FTLD-U represent two extremes of a clinicopathological spectrum [132]. Thus, understanding the nuclear pathology of FTLD-U has important implications with respect to elucidating the pathophysiology of ALS (see below).

We demonstrated that a subset of patients with FTLD-U harboured, in addition to cytoplasmic inclusions, ubiquitinated, round-, rod-, or lens-shaped inclusions within the nucleus [133]. NIs in FTLD-U also show immunostaining for SUMO-1 [134] and variable immunoreactivity to heat shock proteins 40 and 70 [135], p62 [135], valosin-containing protein (VCP) and PML [132,134,135]. Notably, NIs are reportedly negative for CREB or CBP [134]. In our original study, all patients with NIs had a family history suggestive of autosomal dominant inheritance [133] and the link between NIs in FTD and familial disease was later confirmed [136]. Linkage analyses of FTLD-U families with NIs ultimately led to elucidation of the genetic basis of FTLD-U with NIs; mutations in the progranulin (*PGRN*) gene [137,138] located a mere 1.7 Mb centromeric of the highly vilified TAU gene. Progranulin is a 593 amino acid growth factor expressed in several tissues which has roles in development, tissue repair, and inflammation [139]. Haploinsufficiency for this growth factor in the CNS has been postulated as the pathogenetic substrate for FTLD-U with NIs. Other genes for FTLD-U have been identified including the chromatin-modifying protein 2B (CHMP2B) on chromosome 3 [140], and within a 9.8 Mb region at chromosome 9p13.2-21.3 [141,142].

Inclusion body myositis/Paget's disease of bone/frontotemporal dementia (IBMPFD) is a rare, autosomal dominant disorder caused by [129] mutations in the gene encoding VCP [143], a member of the AAA-ATPase gene superfamily (*A*TPase *A*ssociated with diverse cellular *A*ctivities) [144,145]. The histopathological basis of FTD in IBMPFD is the presence of ubiquitinated intranuclear inclusion bodies in cortical neurons as well as dystrophic neurites and only rare cytoplasmic inclusions.

In contradistinction to the nuclear inclusion diseases discussed above, the NIs in FTLD-U and IBMPFD do not appear to be composed primarily of the protein which is mutated. Instead, the principle constituent of the NIs, as well as the cytoplasmic inclusions, in these disorders is transactive response DNA binding protein 43 (TDP-43; Fig. 5) [146,147]. As predicted, the cytoplasmic inclusions in sporadic ALS are also immunoreactive for TDP-43 [146]. The TDP-43 within NIs and cytoplasmic inclusions in these "TDP-43 proteinopathies" displays a characteristic biochemical signature whereby it consists of hyperphosphorylated and ubiquitinated carboxy-terminal fragments [146]. How *PGRN* or *VCP* mutations culminate in aberrant TDP-43 cleavage, phosphorylation, ubiquitination, and aggregation is uncertain. Interestingly, it has been demonstrated that progranulin mediates the caspase-dependent cleavage of TDP-43, providing a possible mechanistic link [148].

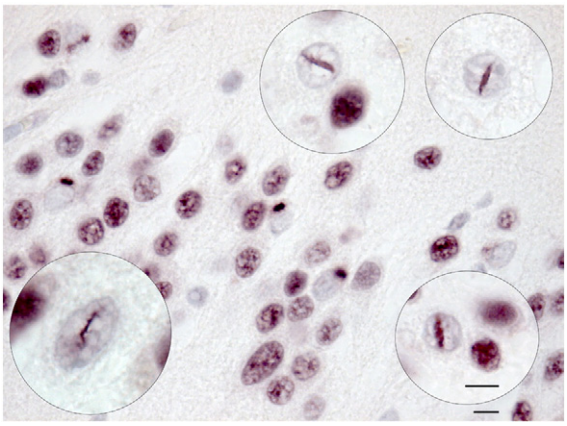

Fig. 5. Dentate gyrus of the hippocampus from a patient with FTLD-U immunostained for TDP-43. Neurons with cytoplasmic (arrowhead) or intranuclear (arrows) inclusions show a loss of the *normal* diffuse nuclear pattern of TDP-43 (shown at higher power for intranuclear inclusions in insets. Bars = 5 μM. Photograph courtesy of Dr. Juan Bilbao.

In normal cells, TDP-43 displays a widespread nuclear distribution. In FTLD-U, IBMPFD, and ALS, NIs and cytoplasmic inclusions exhibit intense TDP-43 staining, at the expense of this pan-nuclear distribution (Fig. 5) [146]. This has been interpreted to suggest that the formation of TDP-43 inclusions sequesters normal TDP-43 from its normal intranuclear location(s), thereby abrogating its function and culminating in neurodegeneration. What function normally mediated by nuclear TDP-43 might be disrupted by this mechanism in FTLD-U? TDP-43 is a highly conserved DNA- and RNA-binding, nuclear protein that functions as a transcriptional repressor and activator of exon skipping [149]. It achieves this through interaction with heterogeneous nuclear ribonucleoproteins, particularly hnRNP A1, A2/B1, A3, and C1/C2 [150]. TDP-43 also interacts with SMN ([151] see below). However, Neumann and colleagues demonstrated no biochemical changes and no alterations in the subcellular pattern of distribution of hnRNPs A1, A2/B1, C1/C2 or SMN in affected brain regions in patients with FTLD-U [152]. In addition, pathological inclusions in FTLD-U did not immunostain for the tested hnRNPs or SMN.

There is evidence that TDP-43 functions as a scaffold for nuclear bodies through its interaction with SMN. Wang and co-workers [151] demonstrated that TDP-43 is localized to multiple discrete subnuclear structures and claimed that these represented a new category of nuclear body, the TDP body (TB). TBs co-localized or overlapped significantly with multiple types of other nuclear bodies including CBs, PML bodies, splicing speckles, and gems. They associated with the latter through an interaction with SMN. Interestingly, TBs often associated with multiple splicing speckles and gems. In addition, a single TB was often identified in association with more than one type of nuclear body including TB-Gem-CB, TB-Gem-POD, TB-speckle-CB, and TB-speckle-PML body combinations. On the basis of these observations, the authors proposed that TBs represent a bridge serving to link nuclear bodies of the same, or different types. For example, by bridging CBs and splicing speckles, they may provide the spatial proximity necessary for coupling snRNP supply with RNA splicing. Abrogation of this nuclear scaffold function of TDP-43 would be predicted to have a profound affect on nuclear body organization with attendant nuclear dysfunction.

5.4. Amyotrophic lateral sclerosis (ALS)

ALS is a progressive neurodegenerative disorder that involves degeneration of the motor system at all levels [153]. Only 5–10% of ALS patients have a positive family history and these usually show an autosomal dominant pattern of inheritance. The remaining 90% of cases seem to occur sporadically. As discussed above, there is considerable overlap between both familial and sporadic FTLD-U and ALS whereby these two disorders represent two extremes of a clinicopathological spectrum [128,132]. Indeed, immunoreactivity of the characteristic cytoplasmic inclusion bodies in ALS for TDP-43 combined with the fact that TDP-43 in ALS displays the pathogenic biochemical signature seen in FTLD-U, suggests that sporadic ALS, like FTLD-U, is a TDP-43 proteinopathy. Indeed a proportion of ALS cases may be caused by mutations in the gene encoding TDP-43 [154]. Thus, the discussion above regarding abnormal TDP-43 function in the nucleus is also pertinent to ALS. On the other hand, evidence from 3-D deconvolution microscopy indicates that TDP-43 might not be the major ubiquitinated target in inclusions in sporadic ALS and that the primary pathogenic protein remains to be identified [155].

Regarding studies of nuclear structure and nuclear bodies per se, in ALS, much remains to be done. NIs distinct in morphology and distribution from those in FTLD-U have been described in cortical and hippocampal pyramidal neurons in otherwise typical cases of ALS [156,157].These round, hyaline structures were immunoreactive for ubiquitin, PML, proteasome, and non-expanded ataxin-3 [157]. In one study, astrocytic PML bodies were implicated in ALS pathophysiology [158]. Excitatory amino acid transporter 2 (EAAT2) is an astrocytic glutamate transporter which under normal conditions is involved in clearing the extracellular space of glutamate and mitigating excitotoxicity. Astrocytic EAAT2 activity is deficient in ALS, possibly as a result of aberrant caspase-mediated cleavage, implicating excitotoxicity in the mechanisms underlying motor neuron degeneration. Gibb and coworkers [158] demonstrated that a caspase-cleaved carboxy-terminal fragment of EAAT2 is SUMOylated and transported to the nucleus where it localized to PML bodies. In light of the involvement of PML bodies in transcriptional regulation, they proposed that the recruitment of SUMOylated EAAT2 carboxy-terminal fragment to PML bodies might result in altered transcription with, for example, increased generation of toxic proteins. In this context, a possible role for PML bodies or other nuclear bodies in ALS warrants further investigation.

6. Conclusions

Ongoing studies of the cell nucleus are revealing a remarkable complexity of structure and function. Underlying this are not only the dynamic interactions among nuclear bodies, emphasized in this review, but also the structural and gate-keeping function of the nuclear membrane, nuclear pores and their associated cytoskeletal complex as well as the non-random localization of the chromosomes themselves to "chromosome territories" [159]. The visually imposing protein aggregates which represent the morphological hallmarks of neurodegenerative disease have perhaps diverted attention away from the nuclear compartment. However, as elucidated above, there is growing recognition that nuclear dysfunction may have an important role in these diseases. Studies of nuclear body alterations in neurodegenerative disease are in their infancy. However, based on what is known, it appears that this may take many forms; the absence of gems in SMA, the disruption of PML bodies and other nuclear bodies in the nuclear inclusion diseases, the potential disruption of TDP-43-mediated linking of nuclear bodies in FTD. For the more common sporadic neurodegenerative disorders, the story remains to be written. However, it is clear that in addition to elucidating novel mechanisms of disease, studying the nucleus in these neurodegenerative disorders could lead to the development of potentially exciting disease-modifying therapies, such as those targeting transcriptional regulation.

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